



**GeneQuery™ Human PD-1/PD-L1 Checkpoint Pathway qPCR Array Kit
(GQH-PDI)
Catalog #GK121**

Product Description

ScienCell's GeneQuery™ Human PD-1/PD-L1 Checkpoint Pathway qPCR Array Kit (GQH-PDI) is designed to facilitate gene expression profiling of 88 key genes involved in human PD-1/PD-L1 checkpoint pathway, and to help identifying potential prognostic biomarkers for cancer immunotherapy. PD-1/PD-L1 checkpoint pathway plays an important role in the maintenance of T cell stability, as well as mediating inhibitory signals for proliferation and function of T effector cells. PD-1/PD-L1 immune checkpoint blockade has resulted in enhancement of T cell immunity against tumors and viral pathogens. Brief examples of how genes may be grouped according to their functions are shown below:

- **Co-stimulatory/inhibitory receptors:** PDCD1 (PD-1), CD28, CD27, CD276, ICOS, TNFRSF18, TNFRSF4, TNFRSF9
- **Co-stimulatory/inhibitory pathway:** CD274 (PD-L1), PDCD1LG2 (PD-L2), CD4, LCK, CD2, CD247, CSK, PTPN11, PTPN2, PTPN6, NCOR2
- **PD-L1 induction:** IFNG, IL15, IL17A, IL2, IL21, IL7, MAPK1, NRP1, STAT3
- **T cell receptor signaling:** AKT1, CD3, CTLA4, CD8, GSK3B, JUN, NFKB1, PDPK1
- **T cell function regulation:** BATF, FOXO1, KRAS, MAPK8, MTOR, NFATC1, PI3Ks, SKP2, TLR9
- **B cell function regulation:** CD80, CD86, POU2F2
- **Proinflammatory molecules:** CSF2, IL4, IL10, IRF6, TNF, VEGFA
- **Additional immune checkpoint genes:** HAVCR2, HNF1A, LAG3, TIGIT

Note: all gene names follow their official symbols by the Human Genome Organization Gene Nomenclature Committee (HGNC).

GeneQuery™ qPCR array kits are qPCR ready in a 96-well plate format, with each well containing one primer set that can specifically recognize and efficiently amplify a target gene's cDNA. The carefully designed primers ensure that: (i) the optimal annealing temperature in qPCR analysis is 65°C (with 2 mM Mg²⁺, and no DMSO); (ii) the primer set recognizes all known transcript variants of target gene, unless otherwise indicated; and (iii) only one gene is amplified. Each primer set has been validated by qPCR with melt curve analysis, and gel electrophoresis.

GeneQuery™ qPCR Array Kit Controls

Each GeneQuery™ plate contains eight controls (Figure 1).

- Five target housekeeping genes (ACTB, GAPDH, LDHA, NONO, and PPIH), which enable normalization of data.
- The Genomic DNA (gDNA) Control (GDC) detects possible gDNA contamination in the cDNA samples. It contains a primer set targeting a non-transcribed region of the genome.

- Positive PCR Control (PPC) tests whether samples contain inhibitors or other factors that may negatively affect gene expression results. The PPC consists of a predisposed synthetic DNA template and a primer set that can amplify it. The sequence of the DNA template is not present in the human genome, and thus tests the efficiency of the polymerase chain reaction itself.
- The No Template Control (NTC) is strongly recommended, and can be used to monitor the DNA contamination introduced during the workflow such as reagents, tips, and the lab bench.

Kit Components

Component	Cat #	Quantity	Storage
GeneQuery™ array plate with lyophilized primers	GK121	1	4°C or -20°C
Optical PCR plate seal	N/A	1	RT
Nuclease-free H ₂ O	GQ100-1	2	4°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
Reverse transcriptase	First-Strand cDNA Synthesis Master Mix, 4x (ScienCell, Cat #MB6008)
cDNA template	Customers' samples
qPCR master mix	GoldNStart TaqGreen qPCR Master Mix (ScienCell, Cat #MB6018)

Quality Control

All the primer sets are validated by qPCR with melt curve analysis. The PCR products are analyzed by gel electrophoresis. Single band amplification is confirmed for each set of primers.

Product Use

GQH-PDI is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped at ambient temperature. Upon receipt, the plate should be stored at 4°C and is good for up to 12 months. For long-term storage (>1 year), store the plate at -20°C in a manual defrost freezer.

Procedures

Note: The primers in each well are lyophilized.

1. Prior to use, allow plates to warm to room temperature.
2. Briefly centrifuge at 1,500x g for 1 minute before slowly peeling off the seal.
3. Prepare 20 µl PCR reactions for one well as shown in Table 1.

Table 1

cDNA template	0.2 – 250 ng
2x qPCR master mix	10 µl
Nuclease-free H ₂ O	variable
Total volume	20 µl

Important: *Only* use polymerases with hot-start capability to prevent possible primer-dimer formation. *Only* use nuclease-free reagents in PCR amplification.

4. Add the mixture of 2x qPCR master mix, cDNA template, and nuclease-free H₂O to each well containing the lyophilized primers. Seal the plate with the provided optical PCR plate seal.

Important: *In NTC control well, do NOT add cDNA template. Add 2x qPCR master mix and nuclease-free H₂O only.*

5. Briefly centrifuge the plates at 1,500x g for 1 minute at room temperature. For maximum reliability, replicates are strongly recommended (minimum of 3).
6. For PCR program setup, please refer to the instructions of the master mix of the user's choice. We recommend a typical 3-step qPCR protocol for a 200nt amplicon:

Three-step cycling protocol

Step	Temperature	Time	Number of cycles
Initial denaturation	95°C	10 min	1
Denaturation	95°C	20 sec	40
Annealing	65°C	20 sec	
Extension	72°C	20 sec	
Data acquisition	Plate read		
<i>Recommended</i>	<i>Melting curve analysis</i>		1
Hold	4°C	Indefinite	1

7. (Optional) Load the PCR products on 1.5% agarose gel and perform electrophoresis to confirm the single band amplification in each well.

Figure 1. Layout of GeneQuery™ qPCR array kit controls.

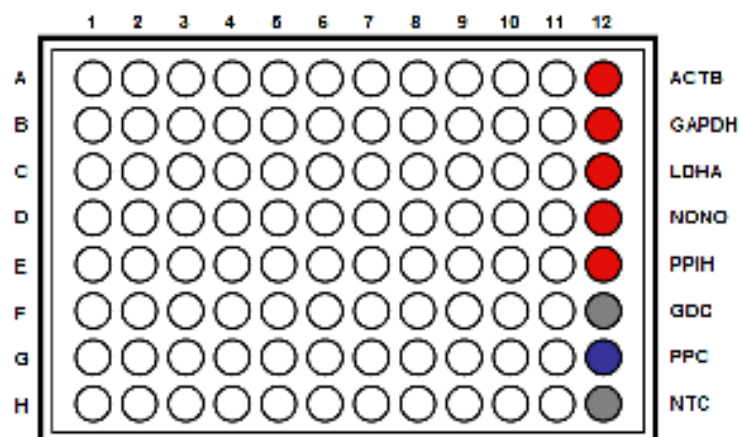


Table 2. Interpretation of control results:

<i>Controls</i>	<i>Results</i>	<i>Interpretation</i>	<i>Suggestions</i>
Housekeeping gene controls	Variability of a housekeeping gene's C _q value	The expression of the housekeeping gene is variable in samples; cycling program is incorrect	Choose a constantly expressed target, or analyze expression levels of multiple housekeeping genes; use correct cycling program and make sure that all cycle parameters have been correctly entered
gDNA Control (GDC)	C _q ≥ 35	No gDNA detected	N/A
	C _q < 35	The sample is contaminated with gDNA	Perform DNase digestion during RNA purification step
Positive PCR Control (PPC)	C _q > 30; or The C _q variations > 2 between qPCR Arrays.	Poor PCR performance; possible PCR inhibitor in reactions; cycling program incorrect	Eliminate inhibitor by purifying samples; use correct cycling program and make sure that all cycle parameters have been correctly entered
No Template Control (NTC)	Positive	DNA contamination in workflow	Eliminate sources of DNA contamination (reagents, plastics, etc.)

Rev.1

Figure 2. A typical amplification curve showing the amplification of a qPCR product.

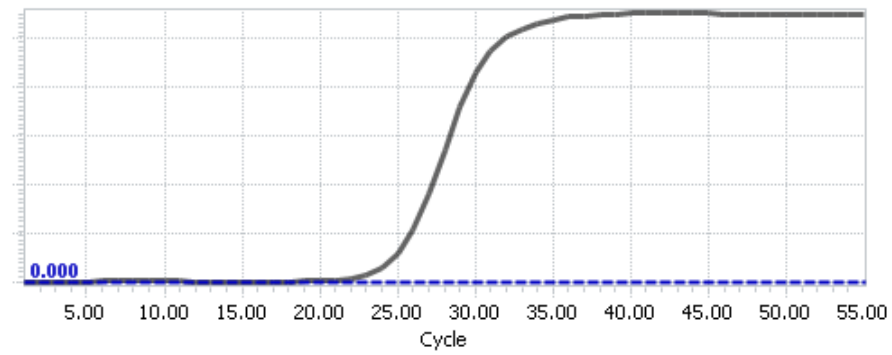
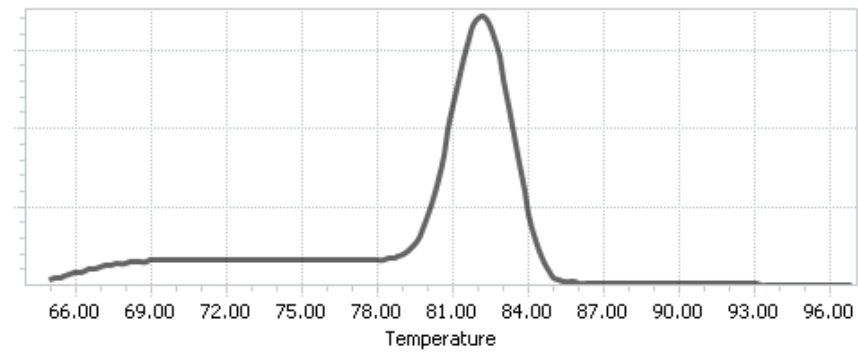


Figure 3. A typical melting peak of a qPCR product.



Quantification Method: Comparative $\Delta\Delta Cq$ (Quantification Cycle Value) Method

1. **Note:** Please refer to your qPCR instrument's data analysis software for data analysis. The method provided here serves as guidance for quick manual calculations.

You can use one or more housekeeping genes as a reference to normalize samples.

Important: We highly recommend using all 5 housekeeping genes included in this kit: ACTB, GAPDH, LDHA, NONO, and PPIH.

2. For a single housekeeping gene, ΔCq (ref) is the quantification cycle number change for that housekeeping gene (HKG) between an experimental sample and control sample.

$$\Delta Cq \text{ (ref)} = Cq \text{ (HKG, experimental sample)} - Cq \text{ (HKG, control sample)}$$

When using multiple housekeeping genes as a reference, we recommend normalizing using the geometric mean [1] of the expression level change, which is the same as normalizing using the arithmetic mean of ΔCq of the selected housekeeping genes.

$\Delta Cq \text{ (ref)} = \text{average } (\Delta Cq \text{ (HKG1)}, \Delta Cq \text{ (HKG2)}, \dots, \Delta Cq \text{ (HKG } n))$ (n is the number of housekeeping genes selected)

If using all 5 housekeeping genes included in this kit (ACTB, GAPDH, LDHA, NONO, and PPIH) use the following formula:

$$\Delta Cq \text{ (ref)} = (\Delta Cq(\text{ACTB}) + \Delta Cq(\text{GAPDH}) + \Delta Cq(\text{LDHA}) + \Delta Cq(\text{NONO}) + \Delta Cq(\text{PPIH})) / 5$$

Note: $\Delta Cq \text{ (HKG)} = Cq \text{ (HKG, experimental sample)} - Cq \text{ (HKG, control sample)}$, and $\Delta Cq \text{ (HKG)}$ value can be positive, 0, or negative.

3. For any of your genes of interest (GOI),

$$\Delta Cq \text{ (GOI)} = Cq \text{ (GOI, experimental sample)} - Cq \text{ (GOI, control sample)}$$

$$\Delta\Delta Cq = \Delta Cq \text{ (GOI)} - \Delta Cq \text{ (ref)}$$

$$\text{Normalized GOI expression level fold change} = 2^{-\Delta\Delta Cq}$$

References

[1] Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. (2002) "Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes." *Genome Biol.* 3(7): 1-12.

Example: Comparative $\Delta\Delta Cq$ (Quantification Cycle Value) MethodTable 3. Cq (Quantification Cycle) values of 2 genes-of-interest and 5 housekeeping genes obtained for experimental and control samples.

Samples	Genes of Interest		Housekeeping Genes				
	GOI1	GOI2	<i>ACTB</i>	<i>GAPDH</i>	<i>LDHA</i>	<i>NONO</i>	<i>PPIH</i>
Experimental	21.61	22.19	17.16	17.84	20.12	19.64	26.40
Control	33.13	26.47	18.20	18.48	20.57	19.50	26.55

$$\begin{aligned}\Delta Cq(\text{ref}) &= (\Delta Cq(\text{ACTB}) + \Delta Cq(\text{GAPDH}) + \Delta Cq(\text{LDHA}) + \Delta Cq(\text{NONO}) + \Delta Cq(\text{PPIH})) / 5 \\ &= ((17.16 - 18.20) + (17.84 - 18.48) + (20.12 - 20.57) + (19.64 - 19.50) + (26.40 - 26.55)) / 5 \\ &= -0.43\end{aligned}$$

$$\begin{aligned}\Delta Cq(\text{GOI1}) &= 21.61 - 33.13 \\ &= -11.52\end{aligned}$$

$$\begin{aligned}\Delta Cq(\text{GOI2}) &= 22.19 - 26.47 \\ &= -4.28\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI1}) &= \Delta Cq(\text{GOI1}) - \Delta Cq(\text{ref}) \\ &= -11.52 - (-0.43) \\ &= -11.09\end{aligned}$$

$$\begin{aligned}\Delta\Delta Cq(\text{GOI2}) &= \Delta Cq(\text{GOI2}) - \Delta Cq(\text{ref}) \\ &= -4.28 - (-0.43) \\ &= -3.85\end{aligned}$$

$$\begin{aligned}\text{Normalized GOI1 expression level fold change} &= 2^{-\Delta\Delta Cq(\text{GOI1})} \\ &= 2^{11.09} \\ &= 2180\end{aligned}$$

$$\begin{aligned}\text{Normalized GOI2 expression level fold change} &= 2^{-\Delta\Delta Cq(\text{GOI2})} \\ &= 2^{3.85} \\ &= 14.4\end{aligned}$$

Conclusion: Upon treatment, expression level of GOI1 increased 2,180 fold, and expression level of GOI2 increased 14.4 fold.



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GeneQuery™ Human PD-1/PD-L1 Checkpoint Pathway qPCR Array Plate Layout*
 (***8 controls*** in Bold and Italic)

Note: all gene names follow their official symbols by HGNC

	1	2	3	4	5	6	7	8	9	10	11	12
A	AKT1	CD276	CD80	FOXO1	HLA-DQB1	IL15	JUN	MAPK8	NRP1	POU2F2	TLR9	<i>ACTB</i>
B	BATF	CD28	CD86	FOXP3	HLA-DRA	IL17A	KRAS	MTOR	PDCD1	PTCRA	TNF	<i>GAPDH</i>
C	BCL2L1	CD3D	CD8A	FYN	HLA-DRB1	IL2	LAG3	NCK1	PDCD1LG2	PTPN11	TNFRSF18	<i>LDHA</i>
D	CALM1	CD3E	CD8B	GSK3B	HNF1A	IL21	LCK	NCOR2	PDPK1	PTPN2	TNFRSF4	<i>NONO</i>
E	CD2	CD3G	CSF2	HAVCR2	ICOS	IL4	LCP2	NFATC1	PIK3CA	PTPN6	TNFRSF9	<i>PPIH</i>
F	CD247	CD4	CSK	HLA-DPA1	ICOSLG	IL7	MAP2K1	NFATC2	PIK3CB	SKP2	TNFSF14	<i>GDC</i>
G	CD27	CD47	CTLA4	HLA-DPB1	IFNG	IRF4	MAP3K7	NFKB1	PIK3CD	STAT3	VEGFA	<i>PPC</i>
H	CD274	CD7	DPP4	HLA-DQA1	IL10	IRF6	MAPK1	NFKBIA	PIK3CG	TIGIT	ZAP70	<i>NTC</i>

* gene selection may be updated based on new research and development

Appendix. Plate type choice chart.

Plate type A

Brand	Model	kit catalog #
ABI / Life Tech	ABI 5700	GK121-A
	ABI 7000	GK121-A
	ABI 7300	GK121-A
	ABI 7500	GK121-A
	ABI 7700	GK121-A
	ABI 7900 HT	GK121-A
	QuantStudio	GK121-A
	ViiA 7	GK121-A
Bio-Rad	Chromo4	GK121-A
	iCycler	GK121-A
	iQ5	GK121-A
	MyiQ	GK121-A
	MyiQ2	GK121-A
Eppendorf / Life Tech	Matercycler ep realplex 2	GK121-A
	Matercycler ep realplex 4	GK121-A
Stratagene	MX3000P	GK121-A
	MX3005P	GK121-A

Plate type B

Brand	Model	kit catalog #
ABI / Life Tech	ABI 7500 Fast	GK121-B
	ABI 7900 HT Fast	GK121-B
	QuantStudio Fast	GK121-B
	StepOnePlus	GK121-B
	ViiA 7 Fast	GK121-B
Bio-Rad	CFX Connect	GK121-B
	CFX96	GK121-B
	DNA Engine Opticon 2	GK121-B
Stratagene	MX4000	GK121-B

Plate type C

Brand	Model	kit catalog #
Roche	Lightcycler 96	GK121-C
	Lightcycler 480 (96-well)	GK121-C